

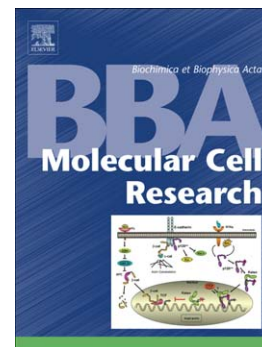
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“Ca²⁺ signaling and cell death”:

**the ECS 2013 workshop in Leuven and a tribute to Humbert De
Smedt**

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In recent years it has become increasingly clear that changes in intracellular Ca^{2+} homeostasis and dynamics are intimately associated with cell death and survival processes, including proliferation, apoptosis, autophagy, and unfolded protein response. As such, different Ca^{2+} transport systems, including channels, transporters and pumps, control these processes. Furthermore, dysregulation of intracellular Ca^{2+} -signaling events and remodeling of Ca^{2+} -transport systems and their regulation by associated proteins and other cellular factors seem to be a hall-mark of several diseases, including different cancer types and neurodegenerative diseases. Understanding the molecular mechanisms underlying these events may now permit novel and unprecedented therapeutic strategies to target these Ca^{2+} -transport systems and complexes.

In this workshop, attracted by more than 100 scientists from 20 different countries, four scientific sessions were organized, dealing with various aspects of Ca^{2+} -signaling in cell survival and cell death. The role of mitochondria, autophagy, ER stress and other factors and their influence on diseases has been discussed. Three contributions were awarded with the best oral communication and best poster prizes by junior scientists. The scientific career, achievements and mentorship of Humbert De Smedt were celebrated at the end of this workshop.

We summarize the lectures of this workshop, for details see the contributions of this special issue and references listed therein.

Opening lecture by Katsuhiko Mikoshiba

The meeting was opened by an impressive keynote lecture given by Katsuhiko Mikoshiba (RIKEN, Japan), who 25 years ago identified the P400 protein as the IP_3 -binding protein which turned out to be an IP_3 -sensitive Ca^{2+} -release channel. He described in detail the critical roles of the different IP_3 R isoforms in development, cell function and diseases. Of special interest was his description of the critical roles of IP_3 R isoforms in cell death, since IP_3 R1 channels are dysfunctional during prolonged ER stress-induced cell death. This has been attributed to an impaired interaction between the IP_3 R1 channel and GRP78, an intraluminal chaperone and key regulator of the unfolded protein response. Very intriguingly, Dr. Mikoshiba discussed his view of IP_3 R as a hub for the interaction with a number of different regulators, especially IRBIT, which coordinates a plethora of ion channels and transporters [1].

Session 1: The complex role of Ca^{2+} signaling in life and death

Dr. Prevarskaya (Lille, France) discussed the regulation of cell death by Ca^{2+} signaling mechanisms in cancer cells, in particular by Ca^{2+} -influx channels. Dr. Prevarskaya described that next to the CRAC channels such as Orai1 different members of the transient receptor potential (TRP) family critically control cell proliferation and apoptosis. Cancer cells can switch their TRP-expression profile to promote oncogenic survival of malignant cells. Her research implicated a critical role for Orai channels in triggering Ca^{2+} influx and subsequent apoptosis. Her group provided evidence that loss of Orai1 expression is one of the key factors that may underlie the appearance of aggressive and apoptosis resistant prostate cancers upon androgen ablation therapy. On the other hand, Prevarskaya and her group discovered that Orai3 is overexpressed in a set of prostate cancer cells

obtained from patients, thereby promoting the proliferation of cancer cells. Finally, Orai1 and Stim1 seem to contribute to the apoptotic resistance of pancreatic adenocarcinoma [2].

Beyond plasmalemmal TRP and Orai channels, it also became evident from the presentation of Dr. Leybaert (Ghent, Belgium) that connexin channels, a conserved family of trans-membrane proteins of which more than 20 isoforms exist in mammals, critically control cell death spreading via gap junction- and hemichannel-dependent functions. Thus, intercellular Ca^{2+} waves are propagated by the passage of IP_3 through those gap junctions triggering Ca^{2+} release from reticular stores in the recipient cells. The spreading of IP_3 is critical but not sufficient for cell death. Hence, additional co-factors are required but still have to be identified. In any case, intercellular Ca^{2+} waves are important for cell death and survival processes in a variety of physiological systems, including the brain, and pathophysiological conditions [3].

Session 2: Ca^{2+} , mitochondria and cell death

In a fascinating lecture Dr. Scorrano (Padova, Italy) described the proteins and mechanisms contributing to tethering complexes between the ER and mitochondria and their impact on cell life and death. Of special interest was his finding that mitofusin 2 (Mfn2), a dynamin-related GTPase, is enriched at ER-mitochondrial contact sites co-localizing with Bax in so-called *foci*. Mfn2-expression levels impact mitochondrial Ca^{2+} transfer and apoptosis. Ablation of Mfn2 in mice is embryonic lethal, Mfn2 mutations in human causes a sensorimotor neuropathy. Mfn2 is also critical for cardiac development and cardiomyocyte differentiation by orchestrating Notch signaling via Ca^{2+} influx and calcineurin activation [4]. In his review, Dr. Scorrano discusses the emerging molecular links and functions of ER-mitochondrial tethers in and beyond Ca^{2+} signaling, thereby controlling cell death and survival [5].

In her lecture Dr. Shoshan-Barmatz (Beer-Sheva, Israel), highlighted the functions of the voltage-dependent anion channel (VDAC) located in the outer mitochondrial membrane. In mammals, three homologous isoforms of similar molecular weights exist, namely VDAC1, 2 and 3. VDAC1 plays a crucial role in regulating metabolic and energetic functions of mitochondria as a high conductance channel for the flux of ATP. VDAC1 also participates in cell survival and death signaling from the mitochondria, e.g. by mediating the release of apoptogenic proteins such as cytochrome C. This is critically dependent on the ability of VDAC1 to form oligomeric structures, which occur in response to a plethora of apoptotic triggers [6].

An interesting view on the role of mitochondria for either cell survival or cell death was offered by Dr. Bernardi (Padova, Italy), who suggested that the old observation of mitochondrial permeability transition leading to matrix swelling of mitochondria may not be an *in vitro* artefact. In his opinion, the observation of the mitochondrial permeability transition leading to an increased permeability of the inner mitochondrial membrane to ions and solutes with molecular weights up to about 1.5 kDa is reflected by opening of a channel, the permeability transition pore (PTP), whose molecular nature remained elusive for a long time [7]. He provided evidence that the PTP is formed by dimerization of the F_0F_1 ATP synthase, since cyclophilin D, a regulator of PTP, binds to the oligomycin sensitivity-conferring protein subunit of the ATP synthase and Bz 423, an inhibitor of the synthase, influences the sensitivity of PTP to Ca^{2+} [8]. Furthermore, purified dimers of the ATP synthase reconstituted into

phospholipid bilayers reflect the electrophysiological properties of PTP. These PTP-like channel events could also be triggered by Bz 423 in the presence of Ca^{2+} . Hence, Dr. Bernardi concluded that the ATP synthase is not only the key enzyme of life, but also promotes signals to cell death.

Session 3: Ca^{2+} and ER stress

Adaptation to and coping with stress is an advantage of cells to struggle for survival. Dr. Michalak (Edmonton, Canada) reviewed in detail how cells can cope with ER stress. The ER is responsible for a wide variety of essential cellular housekeeping functions, among them the synthesis, folding, posttranslational modification, quality control and transport of proteins, and the uptake, storage and release of intracellular Ca^{2+} to keep the cellular calcium homeostasis and dynamics in balance. Hence, disturbed ER functions and concomitant ER stress leads to the coordinated activation of the unfolded protein response (UPR), to correct and cope with the ER stress or to eliminate cells with irreparable ER stress. Three signaling proteins are important for UPR: i) IRE1 α , an inositol-requiring endonuclease responsible for the unconventional splicing of XBP-1, but also for the degradation of RNAs including microRNAs, ii) PERK, a protein kinase RNA-like ER kinase that phosphorylates eukaryotic translation initiation factor 2 α (eIF2 α); iii) ATF6, a transcription factor that translocates to the Golgi and is activated by proteolytic cleavage. Under normal conditions IRE1 α , PERK and ATF6 are inactive due to their interaction with the ER chaperone BiP. Upon activation of UPR, BiP is released from IRE1 α , PERK and ATF6, coordinating the expression of ER chaperones and anti-oxidants, ER-associated protein degradation, autophagy and apoptosis. Thus, the orchestrated action of IRE1 α , PERK and ATF6 permits the cell to cope with ER stress and is an integral part of normal physiology, either to ensure cell survival or to eliminate damaged cells. This system is fundamental for the health of virtually all cells and tissues, including components of the cardiovascular system. The role and status of the UPR is also emerging being important for the survival of cancer cells [9].

Dr. Kaufman (La Jolla, USA), one of the pioneers of UPR research and its impact on human disease, related Ca^{2+} signaling to protein folding through mitochondrial function. PERK-mediated phosphorylation of eIF2 α on the one hand leads to the translation of ATF4 mRNA and on the other hand leads to a block in translation and general protein synthesis. Under mild or short-term ER stress, ATF4 promotes survival by upregulating a subset of adaptive UPR executioners, including foldases, oxidoreductases and proteins critical for autophagy induction. However, during prolonged or excessive ER stress, ATF4 also induces the transcription of CHOP, a long-known component of the pro-apoptotic UPR arm. Dr. Kaufman showed that the building of the ATF4/CHOP complex as a heterodimer is required to induce their target genes and promote UPR. On the other hand, in response to increased protein misfolding in the ER Ca^{2+} leaks from the ER, thereby causing mitochondrial damage and increasing the probability of cell death [10].

Session 4: Ca^{2+} , endoplasmic reticulum and cell death

According to Dr. Foskett (Philadelphia, USA), anti-apoptotic proteins such as Bcl-2 or Bcl-XL regulate the release channel activity of IP₃ receptors by directly interacting with the C-terminal tail of IP₃R to

sensitize single channels in the ER to low IP₃ concentrations. Using patch clamp experiments with isolated nuclei from Sf9 cells expressing only IP₃R1, it was convincingly shown that Bcl-XL in the absence of IP₃ could not induce channel opening, but prominently sensitized IP₃R channel activity in response to physiological concentrations of IP₃. This mechanism leads to pro-survival Ca²⁺ oscillations that enhance mitochondrial bio-energetics and enable cells to resist apoptosis. Furthermore, this pathway seems an integral part of the anti-apoptotic effect of Bcl-XL in cells, since Bcl-XL overexpression was less effective in protecting against apoptosis in cells lacking IP₃Rs versus cells expressing either one of the IP₃R isoforms. Very recently, it was found that the IP₃R/Bcl-XL nexus is targeted and counteracted by PKC-phosphorylated K-ras, resulting in deficient ER-mitochondrial Ca²⁺ transfer [11]. This is needed for efficient respiration, excessive autophagy activation and subsequent cell death. At the level of the mitochondria, it becomes increasingly clear that the recently identified mitochondrial Ca²⁺ uniporter (MCU) is involved in mediating Ca²⁺ uptake to stimulate ATP production and thus suppressing basal autophagy. The essential role of MCU in these processes is further supported by the finding that ablating mitochondrial calcium uniporter regulator 1 (MICUR1), an essential regulator of MCU-driven mitochondrial Ca²⁺ uptake, abrogates oxidative phosphorylation, impairs the production of ATP and triggers AMP kinase-dependent autophagy as a pro-survival rescue mechanism [12].

Dr. Distelhorst (Cleveland, USA) explained the role of anti-apoptotic Bcl-2 proteins executing their role as cellular inhibitors of the Ca²⁺-flux properties of IP₃R channels. This interaction can be targeted using peptide tools that mimic the Bcl-2-binding site of IP₃Rs. Dr. Distelhorst described that in T cells Bcl-2 was essential to recruit DARPP-32 and calcineurin, the Ca²⁺/calmodulin-dependent phosphatase PP2B, to IP₃R. This complex serves as a critical regulator of IP₃R-dependent Ca²⁺ release important for the survival of cells, including primary chronic lymphocytic leukemia cells and is proposed as a new target in anti-cancer strategies [13].

Finally, Dr. Hajnoczky (Philadelphia, USA) reported his results concerning one of the hot topics of the workshop, namely the junctions formed by mitochondria with the endoplasmic reticulum. He described these junctions as „quasi synaptic Ca²⁺ signaling be-tween ER and mitochondria”, which can be biochemically isolated as mitochondrial ER-associated membranes. These junctions play a pivotal role in mediating calcium signal propagation to mitochondria, important for ATP production, a life signal, and mitochondrial cell death. Elegant tools have been developed by his group to chemically establish ER-mitochondrial contact sites, to visualize ER-mitochondrial contact sites and to measure Ca²⁺ in the microdomain of the ER-mitochondrial contact sites [14]. Many of the ER-mitochondria Ca²⁺ signaling events are sensitive to redox changes, probably exposed to reactive oxygen species (ROS) produced by mitochondria and/or the ER. Using a FRET-based strategy, Dr. Hajnoczky and his co-workers were able to measure local Ca²⁺-ROS interactions at the ER-mitochondria interface and provided evidence for its relevance of the function of the mitochondria in both, cell survival and cell death. Recent work led to the determination of MICU1, as a critical regulator of MCU [15]. MICU1 critically determines the threshold and cooperativity of MCU-mediated Ca²⁺ transport in the mitochondria. Ablating MICU1 enhances the uptake of low-level Ca²⁺ signals, but dampens the uptake of larger Ca²⁺ signals that were triggered by agonists.

The scientific program was ended by Dr. De Smedt, who was a mentor and inspirer for many of us at the KU Leuven (Fig. 1). He inspired Jan B. Parys, Ludwig Missiaen and Geert Bultynck and numerous PhD students and post-doctoral researchers by his work on IP₃R channels. He summarized twenty years of research in a 40 minutes lecture. His work has led to key discoveries about the regulation of IP₃Rs by intracellular Ca²⁺ and redox systems. Furthermore, one of the key concepts put forward by Dr. De Smedt was that IP₃R channels act as multi-protein complexes and are tightly regulated by a variety of proteins.

One of the key observations made by Dr. De Smedt long time ago concerned the isoform-expression profile of IP₃Rs in different cell types and tissues (Fig. 2). In addition, cell lines in culture seem to be able to switch their IP₃R isoform-expression profile depending on the conditions. Fig. 2 presents a schematic version explaining these ideas and visualizing the expression levels of the three IP₃R isoforms in various tissues and cell types. This concept supports the finding that distinct IP₃R isoforms play very diverse roles in cell-fate decisions [16].

Three awarded contributions by junior scientists

The best short oral communication, donated by the journal BBA-Molecular Cell Research, was given to Nicola Darling (Cambridge, UK) for her work entitled: “ERK1/2 signaling protects against ER stress-induced apoptosis” [17].

One poster prize, donated by the Biochemical Journal, was given to Alex van Vliet (Leuven, Belgium) for his poster entitled: “PERK is a component of the mitochondria-associated membranes that shape ER-mitochondria interactions during ER stress” [18].

Another poster prize, donated by Abcam[®], was given to Dr. Haidar Akl (Leuven, Belgium) for his poster entitled: “Transforming the constitutively active IP₃ signaling in DLBCL and CLL into deadly Ca²⁺ signals by targeting IP₃R/Bcl-2 complexes” [19].

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Figure legends



Figure 1. A picture of Humbert De Smedt, taken at the PhD defense of his last PhD student (13/12/2012).

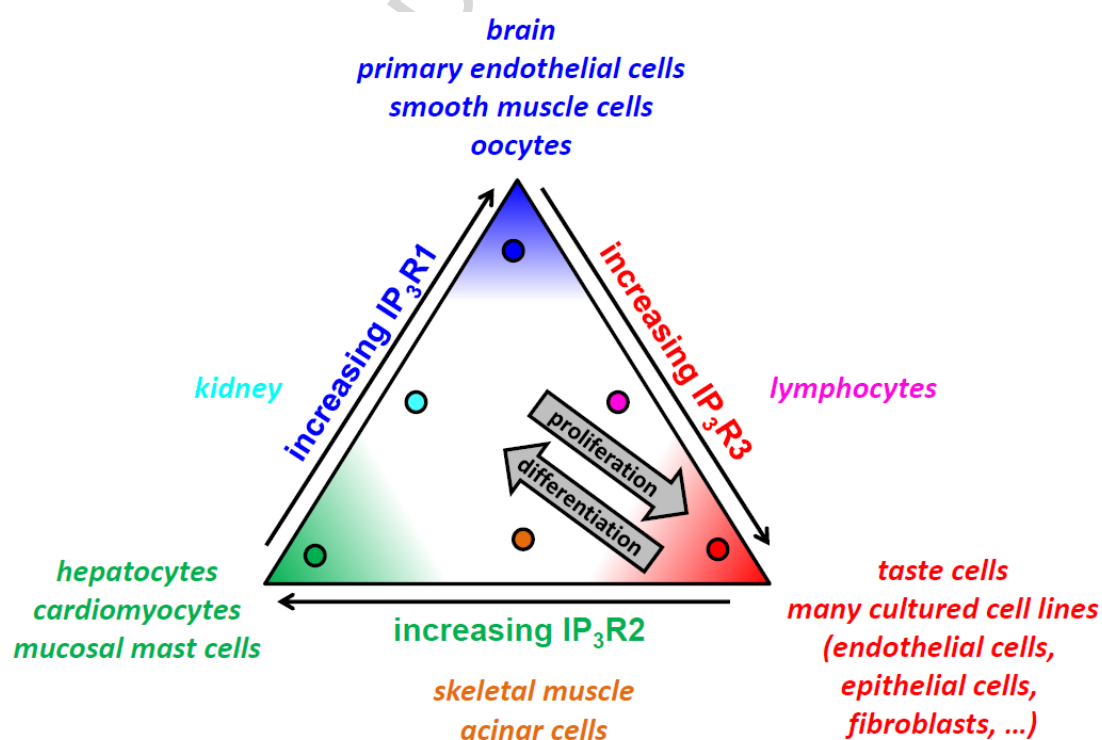


Figure 2. A triangle presentation of the relative expression levels of the three different IP_3R isoforms. The blue corner represents high IP_3R1 levels, the green corner represents high IP_3R2 levels, the red corner represents high IP_3R3 levels. Different tissues and cell types display different IP_3R -isoform-expression profiles, which seem to be dynamically regulated during cell proliferation and

differentiation. The figure is inspired by the original figure made by Humbert De Smedt and presented in its original form in this issue [16].

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